Bioactive benzylisoquinoline alkaloids from *Artabotrys hexapetalus*

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**ABSTRACT**

Two new benzylisoquinoline alkaloids, namely, hexapetalines A (1) and B (2), along with 11 known alkaloids were isolated from the stems of *Artabotrys hexapetalus*. The structures of 1 and 2 with their absolute configurations were elucidated by extensive spectroscopic methods and the known compounds were identified by comparisons with data in the literature. All new compounds were evaluated for their cytotoxocities against five human cancer cell lines: HL-60, SMMC-7721, A-549, MCF-7 and SW480 in vitro. Alkaloids 1 and 2 exhibited inhibitory effects with IC\(_{50}\) values comparable to those of cisplatin.

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1. Introduction

The genus *Artabotrys* (Annonaceae) comprising about 100 species grows mainly in tropical and subtropical regions (Chatrou et al., 2004). There are about four species of this genus in China (*Artabotrys hexapetalus*, *Artabotrys pilosis*, *Artabotrys hainanensis* and *Artabotrys hongkongensis*), among which, *A. hexapetalus* is widely distributed throughout the southern part of China. As a Chinese traditional folk medicine, its roots and fruits are used for treating malaria and scrofula, respectively (Radloff et al., 1996; Chatrou et al., 2004; Nicolaou et al., 1994). Previous chemical studies on this genus have led to the isolation of an array of compounds including compounds classified as bisabolene (Liang et al., 1979; Zhang et al., 1988) and guaiane sesquiterpenes (Fleischer et al., 1997), steroids (Hasan et al., 1987), aporphine (Eloumi-Ropivia et al., 1985; Wu et al., 1989; Guinaudeau et al., 1994; Wijeratne et al., 1995; Hsieh et al., 1999) and tetracydroberberine (Cave et al., 1986) alkaloids, long chain hydrocarbons (Jain et al., 1998), and flavonoid glycosides (Li and Yu, 1997; Li et al., 1997). Earlier chemical and pharmacological studies had showed that its sesquiterpenes and alkaloids account for the antimalaria and antitumor activities, respectively. Our preliminary experimental results showed that the alkaloidal extract of the stems of *A. hexapetalus* exhibited significant cell growth inhibition activities against various human cancer cell lines with IC\(_{50}\) in the range of 8.68–28.36 \(\mu\)M in vitro. As a part of our ongoing research into structurally and biologically interesting alkaloids from tropical medicinal plants in China, a chemical investigation on the alkaloidal extract was thus undertaken and has led to the isolation and characterization of two new benzylisoquinoline alkaloids, hexapetalines A (1) and B (2), together with 11 known alkaloids. Their structures and the absolute configurations of hexapetalines A (1) and B (2) were elucidated by extensive spectroscopic methods. All new compounds were evaluated for their cytotoxicities against five human cancer cell lines in vitro. Herein, we describe the isolation, structure elucidation, and cytotoxic properties of these new alkaloids.

2. Results and discussion

The alkaloidal extract of *A. hexapetalus* was separated as described in Section 3.3 to yield a total of 13 alkaloids, including two new ones (as shown in Fig. 1). All of the compounds probably belong to the class of alkaloids, as they were positive in a reaction with Dragendorff’s reagent.

Hexapetaline A (1), obtained as a yellowish amorphous powder with a specific rotation of +88.6 (c 0.11, MeOH), possessed a molecular formula of C\(_{20}\)H\(_{22}\)NO\(_5\), as established by HRESIMS (m/z
absorption at 1H N–CH3 3.21

b Measured 0.685 131.4

d -OCH3 3.15

were 1) = 12.4, 9.8 Hz), 4.03 (1H, dd, J = 12.4, 2.6 Hz), and 2.85 (1H, dd, J = 12.4, 9.8 Hz). All these indicated that alkaloid 1 possesses a benzylisoquinoline skeleton (Zhou et al., 1991; Cui et al., 2007).

Table 1

<table>
<thead>
<tr>
<th>Position</th>
<th>Hexapetaline A (1)</th>
<th>Hexapetaline B (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.46 (1H, dd, J = 9.8, 2.6)</td>
<td>4.10 (1H, dd, J = 10.8, 2.4)</td>
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<tr>
<td>3α</td>
<td>3.46 (1H, m)</td>
<td>3.40 (1H, m)</td>
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<td>3β</td>
<td>3.68 (1H, m)</td>
<td>3.76 (1H, m)</td>
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<tr>
<td>4α</td>
<td>2.92 (1H, m)</td>
<td>3.12 (1H, m)</td>
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<tr>
<td>4β</td>
<td>2.96 (1H, m)</td>
<td>3.16 (1H, m)</td>
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<td>5</td>
<td>6.74 (1H, s)</td>
<td>6.76 (1H, s)</td>
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<tr>
<td>6</td>
<td>148.9 s</td>
<td>149.4 s</td>
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<tr>
<td>7</td>
<td>128.1 s</td>
<td>127.2 s</td>
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<tr>
<td>8</td>
<td>6.31 (1H, s)</td>
<td>5.77 (1H, s)</td>
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<tr>
<td>8α</td>
<td>115.5 s</td>
<td>115.8 s</td>
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<tr>
<td>9</td>
<td>127.1 s</td>
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<td>10</td>
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<tr>
<td>11</td>
<td>172.2 d</td>
<td>118.0 d</td>
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<tr>
<td>3</td>
<td>147.6 s</td>
<td>147.6 s</td>
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<tr>
<td>4</td>
<td>148.1 s</td>
<td>148.0 s</td>
</tr>
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<tr>
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<td>3.83 (3H, s)</td>
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<tr>
<td>N-CH3</td>
<td>3.21 (3H, s)</td>
<td>54.8 q</td>
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</table>

* Measured at 400MHz.

* Measured at 100MHz.
also absolute alkaloids oxide. 

A ½ (+)-1 ydine was determined by HRESIMS (m/z 346.1658, [M+H]+, calcd: 346.1654) and 13C NMR spectroscopic data (Table 1), indicating nine degrees of unsaturation. Comparison of the NMR data of 2 (Table 1) with those of 1 suggested that both alkaloids shared the same basic skeleton, but the molecular weight of 2 is smaller than that of 1 by 14 units. This indicated that the methyl group resonated at δH 3.21 (3H, s) and δC (54.8) at N-2 in 1 was replaced by an atom in 2. Analysis of the 2D NMR data confirmed that the remainder of the structure of 2 was the same as that of 1. Thus, the structure of hexapetaine B (2) was established as the N-demethyl analog of hexapetaine A (1) as shown in Fig. 1.

Hexapetaines A (1) and B (2) both possess two chiral centers at C-1 and N-2, which is identical to (+)-15, 25-laudanidine-N1-oxide, and (+)-15, 2R-laudanidine-N2-oxide. Given that (+)-15, 25-laudanidine-N1-oxide and (+)-15, 2R-laudanidine-N2-oxide hold the same 2D rotation values (δC 139 for 15, 25-laudanidine-N1-oxide and δC 139 for 15, 2R-laudanidine-N2-oxide) (Zhang et al., 2012), we could draw a conclusion that the rotation values of this type of benzylisoquinoline alkaloids mainly depend on the chiral center at C-1. From structural and biogenetic point of view, (+)-reticuline (3), also isolated from this plant by us, whose structure and absolute configuration had been determined by a combination of spectroscopic methods (Arbain et al., 1990; Cui et al., 2007; Blanchfield et al., 2003), hold the same chiral center at C-1 with that of alkaloids 1 and 2, and could be considered as model compound for assignment of the absolute configuration by comparison of rotation value. Hence, the absolute configurations at C-1 of alkaloids 1 and 2 were S-configuration, identical to that of (+)-reticuline, as determined by their similar specific rotation with 3 (δC 139 + 63.9). Another chiral center at N-2 in alkaloids 1 and 2 was determined by the difference in chemical shift for H-8 in 1 and 2, caused by the orientation of the N-oxide. The signal of H-8 in 1 appears relatively downfield compared to that in 2 (δH 6.31 vs 5.77) indicated the α-orientation of the oxygen in compound 1 and the β-orientation of the oxygen in compound 2 (Lee et al., 2007; Zhang et al., 2012).

In addition to benzylisoquinoline alkaloids 1–3, 10 other known aporphinoid alkaloids were isolated and identified as nosoricodyline (4) (Haynes et al., 1966), normuciferine (5) (Shakirov et al., 1996), stepharine (6) (Haynes et al., 1966), isocorydine (7) (Shakirov et al., 1996), anonaine (8) (Sette et al., 2000), roemerine (9) (Sette et al., 2000), liriodenine (10) (Costa et al., 2011), atherospermideine (11) (Costa et al., 2011), laureline (12) (Roblot et al., 1983), arbatonine B (13) (Hsieh et al., 1999), by comparing the experimental and reported physical data.

Hexapetaines A (1) and B (2) were evaluated for their cytotoxicities against five human cancer cell lines, HL-60, SMCC-7721, A-549, MCF-7, and SW480 by the MTT method. Hexapetaine A (1) showed a significant cell growth inhibitory activity against all cell lines with IC50 values at 6.97 ± 0.18, 8.06 ± 0.19, 16.28 ± 0.22, 10.18 ± 0.42, and 13.22 ± 0.28 μM, respectively. Hexapetaine B (2) showed a moderate cell growth inhibitory activity against all cancer cell lines with IC50 values at 14.34 ± 0.14, 18.66 ± 0.28, 28.98 ± 0.16, 26.22 ± 0.33 and 21.58 ± 0.28 μM, respectively. While the cisplatin control showed cytotoxicity with IC50 values at 1.28 ± 0.08, 16.88 ± 0.28, 10.22 ± 0.44, 21.65 ± 0.22, and 25.26 ± 0.46 μM, respectively.

Two new benzylisoquinoline alkaloids, hexapetaines A (1) and B (2), as well as 11 known alkaloids, were isolated from the stems of A. hexapetals. The discoveries of compounds 1 and 2 are not only a further addition to diverse and complex array of benzylisoquinoline alkaloids, but also, their presence as characteristic markers may be helpful in chemotaxonomical classifications. The cytotoxicity against several human cancer cell lines of isolated compounds was also investigated, and found to be quite potent.

3. Experimental

3.1. General experiment procedure

Optical rotations were measured with a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were recorded on a Nicolet Nexus 470 spectrophotometer in KBr discs. NMR spectra were recorded on Bruker 400 MHz spectrometers using TMS as an internal standard, with chemical shifts recorded as δ values. HRESIMS spectra were measured on a Micromass Q-TOF Ultima Global GAA076 LC mass spectrometer. HRESIMS spectra were measured on a Micromass El-4000 (Autospec-Ultima-TOF). Silica gel (300–400 mesh, Qingdao Marine Chemical Inc., China), Silica gel H (10–40 μm, Qingdao Marine Chemical Inc., China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and Sephadex LH-20 (40–70 μm, Amersham Biosciences, Sweden) were used for column chromatography (CC).

Fig. 2. Selected 2D NMR correlations for hexapetaine A (1).
3.2. Plant material

The stems of *A. hexapetalus* (16 kg) were collected from Bawangling Nature Reserve, Hainan Province, China, in August 2012, and identified by Prof. Qiong-Xin Zhong, College of Life Science, Hainan Normal University. A voucher specimen (No. CHEN20120518) has been deposited at the Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University.

3.3. Extraction and isolation

The air-dried stems of *A. hexapetalus* (16 kg) were extracted with CH$_3$OH, and the pH of the crude extract was adjusted with saturated tartaric acid to 2. The acidic mixture was defatted with petroleum ether (PE) and then extracted with CHCl$_3$. The aqeous phase was basified to pH 10 with saturated Na$_2$CO$_3$ and then extracted with CHCl$_3$ to obtain crude alkaloids. The crude alkaloids (78.0 g) were separated on a silica gel column (20–300 mesh; CHCl$_3$/CH$_3$OH, 1:0 → 0:1) and a Sephadex LH-20 column. The fractions were further separated using pre-HPLC (CH$_3$OH/H$_2$O, 65:35) to give 6 (13 mg) and 7 (12 mg). Fraction 3C (2.2 g) was separated on a silica gel column (300–400 mesh; CH$_3$OH/H$_2$O, 10:1) giving three fractions (Fr 2C1–2C3). Fraction 2C1 (68 mg) was purified using a Sephadex LH-20 column eluted with CH$_3$OH/H$_2$O, followed by semi-preparative HPLC using an Waters XBridge C$_{18}$ (10 × 250 mm, 5 μm) column with 50% CH$_3$OH/H$_2$O to afford compounds 2 (19 mg), and 11 (22 mg). Compounds 9 (116 mg) and 10 (18 mg) were obtained from fraction 2C2 (488 mg) by semi-preparative HPLC using a Waters XBridge C$_{18}$ (10 × 250 mm, 5 μm) column with 40% CH$_3$OH/H$_2$O.

3.4. Hexapetaline A (1)

Yellowish amorphous powder; [α]$_D^{+}$ + 88.6 (c 0.11, CH$_3$OH); UV (CH$_3$OH)$_2$ max (log ε) 236 (4.14), 281 nm (3.17); IR (KBr) ν$_{max}$ 3439, 2927, 1634, 1606 and 1456 cm$^{-1}$; 1 H and 13C NMR data (Table 1); ESI-MS m/z 360 [M+H]$^+$; HRESIMS m/z 360.1818 (M+H$^+$; calc for C$_{19}$H$_{26}$NO$_5$ 360.1811).

3.5. Hexapetaline B (2)

Yellowish amorphous powder; [α]$_D^{+}$ + 78.6 (c 0.12, CH$_3$OH); UV (CH$_3$OH)$_2$ max (log ε) 238 (4.34), 286 nm (3.28); IR (KBr) ν$_{max}$ 3422, 2926, 1651, 1605 and 1516 cm$^{-1}$; 1 H and 13C NMR data (Table 1); ESI-MS m/z 346 [M+H]$^+$; HRESIMS m/z 346.1658 (M+H$^+$; calc for C$_{19}$H$_{26}$NO$_5$ 346.1654).

3.6. Cytotoxicity bioassays

The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, MCF-7, and SW480. All cells were cultured in RPMI-1640 or DMEM medium (HyClone, Logan, UT, USA), supplemented with 10% fetal bovine serum (HyClone) in 5% CO$_2$ at 37 °C. The cytotoxicity assay was performed using the MTT method in 96-well microplates (Mosmann, 1983). Briefly, adherent cells (100 μL) were seeded into each well of 96-well cell culture plates and allowed to adhere to 12 h before drug addition, and suspended cells were seeded just before drug addition with an initial density of 1 × 10$^5$ cells/mL. Each tumor cell line was exposed to the tested compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicate for 48 h. Cisplatin (Sigma, St. Louis, MO, USA) was used as a positive control. After treatment, cell viability was measured and the cell growth curve was plotted. IC$_{50}$ values were calculated by the Reed and Muench method (Reed and Muench, 1938).

**Conflict of interest**

The authors declare that there are no conflict of interest.

**Acknowledgements**

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**References**


